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PATENT & TRADEMARK OFFICE

DOCKET NO. 5470-220CTXX

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: ROBERT E. JOHNSTON, ET AL. ART UNIT:
SERIAL NO.: 10/683,781 EXAMINER:

FILING DATE: OCTOBER 10, 2003
FOR: ALPHAVIRUS RNA REPLICON SYSTEMS

DECLARATION OF NANCY L. DAVIS, PH.D. and ROBERT E. JOHNSTON, PH.D., 37 C.F.R. §1.608(b)

ASSISTANT COMMISSIONER FOR PATENTS
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SIR:

We, Nancy L. Davis, Ph.D. and Robert E. Johnston, Ph.D., do hereby declare and state:

1. We are virologists specializing in vaccine development and have worked together in the area of vaccine development for nearly 20 years in the Department of Microbiology and Immunology at the School of Medicine of the University of North Carolina at Chapel Hill (UNC-CH). At UNC-CH we contributed, through our investigative work, to developing the Alphavirus RNA Replicon Systems of U.S. Patent Application Serial No. 10/683,781. In preparing this declaration we reviewed the following documents:

Exhibit E	Letter from Dr. Nancy Davis to Dr. Jonathan Smith, July 7, 1992
Exhibit F	Letter from Dr. Nancy Davis to Dr. Jonathan Smith, May 24, 1993
Exhibit V	Letter from Dr. Nancy Davis to Dr. Jonathan Smith, May 24, 1992

2. Our involvement (concept development and laboratory research) in developing the Alphavirus RNA Replicon System of U.S. Patent Application Serial No. 10/683,781 ("the '781 application"), and in particular, the subject matter of Claim 116, is discussed herein below. We

understand that the time period spanning from November 30, 1994 through May 23, 1995 is being referred to as the Activity Time Period, however, our contributions occurred prior to and during the Activity Time Period.

3. The Alphavirus RNA Replicon System was conceived well prior to November 30, 1994, and we contributed to formulating the original ideas about how to make and utilize the system as a vaccine. The fundamental and detailed concept of the project was developed during discussion with collaborating scientists Michael Parker ("Parker") and Jonathan Smith ("Smith") of the Virology Division at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID). We understand that Parker and Smith are designated co-inventors of the subject matter of the '781 application and Claim 116.

4. Correspondence sent to Smith at USAMRIID reflects discussions we and others, including at least Parker, had prior to November 30, 1994 regarding the development of the Alphavirus RNA Replicon System. The alphavirus that is described in this correspondence is the Venezuelan Equine Encephalitis virus, referred to as "VEE." (Exhibits E, F, and V).

5. We understand that pending Claim 116 of the '781 application reads as follows:

Claim 116. A method of producing an immune response in a subject, comprising:

administering to the subject an effective amount of a composition comprising a population of infectious, defective alphavirus particles in a pharmaceutically acceptable carrier,

wherein each particle comprises one or more heterologous RNA sequence(s) encoding an immunogen; and further wherein the population contains no detectable replication-competent alphavirus particles as determined by passage on permissive cells in culture.

6. In order to produce the infectious replication defective alphavirus particles required to practice Claim 116, it was necessary to first develop helper and replicon constructs because both

are required to produce a replication-defective RNA-containing infectious particle. The replicon lacks a complete complement of functional alphavirus structural protein genes, and therefore, it cannot produce particles and initiate a productive infection. Similarly, the helper preferably lacks a full complement of functional alphavirus nonstructural protein sequences required for RNA replication and transcription and does not produce RNAs that can be packaged into new virions. As an additional safety measure, we decided to incorporate attenuating mutations into the replicon particles so that in the event that a replication-competent virus was generated (*e.g.*, by homologous recombination), it would not be fully virulent. Thus, we began identifying the attenuated VEE clones developed in our laboratory for use in producing helper and replicon RNAs.

7. Following numerous discussions with Parker and Smith, a letter (signed by Davis) was written, in which the ideas are outlined for how to proceed in constructing VEE replicon and helper RNAs. (Exhibit V, letter of May 24, 1992). This letter includes schematic drawings of the VEE genome segments to be included in the helper and replicon plasmids. Specifically, this letter shows the helper plasmid as including attenuated, but intact, structural genes whose expression would be driven by a cloned 26S subgenomic promoter (Exhibit V, top drawing). In addition, the deletion of the nonstructural genes is shown (Id.) and the VEE clone pV3014 that contains a mutation in the E2 gene is identified for use in constructing the helper (Id., 1st ¶). pV3014 is one of the attenuated VEE clones disclosed in the '781 application.

8. This same letter diagrams the replicon plasmid, showing intact nonstructural protein genes, a cloned 26S promoter, and deletion of the native 26S promoter and structural protein genes. (Exhibit V, bottom drawing). A multiple cloning site (MCS) is also shown, which is the intended site for introducing a heterologous RNA sequence(s) encoding an immunogen of choice

(e.g., Lassa fever nucleoprotein (LSN) antigen). The MCS was included to facilitate cloning of heterologous RNAs by introducing some cleavage sites that are not present in the native VEE replicon sequence (Id., last ¶). This letter documents that a replicon would be produced in our lab and sent to USAMRIID (Id.).

9. Our written correspondence further documents the exchange of attenuated VEE virus clones that we created at UNC-CH and that were subsequently used at USAMRIID to construct VEE replicon and helper RNAs, including those disclosed in the '781 application.

10. For example, in July of 1992 we sent several VEE-derived DNA constructs to USAMRIID, including a VEE clone containing a duplicate (cloned) 26S promoter and three attenuating mutations in the VEE structural genes (pV3507 background). This clone, designated 2-20, is described in the '781 application (Exhibit E, letter to Smith from Davis, July 7, 1992).

11. A shipment of biological materials was sent to Smith at USAMRIID on May 24, 1993. The letter accompanying this shipment (Exhibit F, Letter from Dr. Nancy Davis to Dr. Jonathan Smith, May 24, 1993) documents that we had produced VEE replicon plasmid pVR2, constructed essentially as described in our earlier correspondence. Additional replicon constructs were sent, including one replicon plasmid containing the HA coding sequence (i.e. heterologous RNA sequence encoding the HA immunogen of influenza virus). A shuttle vector comprised of attenuated VEE structural protein genes, a cloned 26S promoter, and a MCS, again as described in previous correspondence, was sent for use in inserting genes into the replicon plasmid. Purified preparations of these constructs and stocks of transformed bacteria were included. (Exhibit F).

12. Prior to and during the Activity Time Period, we routinely (at least weekly) engaged in discussions with our collaborators at USAMRIID, primarily Smith. Our discussions pertained

not only to the development of the replicon and helper sequences, but to developing protocols and assays required for expressing and evaluating the replicon and helper RNAs. These included transfection protocols for efficiently introducing the RNAs into cells (e.g., electroporation protocols) and protocols to confirm the production of infectious but replication defective particles. (e.g., passage on permissive cells).

13. Prior to November 30, 1994, we were aware that Peter Pushko ("Pushko") joined Smith's laboratory at USAMRIID specifically to work on the Alphavirus Replicon RNA Systems and we were aware of his contributions related to constructing helper sequences and producing infectious replication deficient VEE particles that expressed the LSN antigen at high levels in infected cells. We were also aware that Pushko created the reagents used in the first animal study, designed and conducted by George Ludwig ("Ludwig"), whom we understand to be a co-inventor.

We, Nancy L. Davis, Ph.D. and Robert E. Johnston, Ph.D., declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statements may jeopardize the validity of U.S. Patent Application Serial No. 10/683,781 or any patent issuing therefrom.

Sept 17, 2004
Date

Nancy L Davis
Nancy L. Davis, Ph.D.

9/17/04
Date

Robert E. Johnston, Ph.D.